Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 6/2002 - PCT - 1	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DE2003/002688	International filing date (*day/month/year*) 09 August 2003 (09.08.2003)	Priority date (*day/month/year*) 13 August 2002 (13.08.2002)
International Patent Classification (IPC) or national classification and IPC C12N 7/04		
Applicant MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 04 February 2004 (04.02.2004)	Date of completion of this report 08 December 2004 (08.12.2004)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/DE2003/002688

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 7-10, as originally filed
pages _____, filed with the demand
pages 1-6, filed with the letter of 30 June 2004 (30.06.2004)

☒ the claims:
pages 5, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages 1-4, filed with the letter of 30 June 2004 (30.06.2004)

☒ the drawings:
pages 1/7-7/7, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2, 5	YES
	Claims	1, 3, 4	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-5	NO
Industrial applicability (IA)	Claims	1-5	YES
	Claims	none	NO

2. Citations and explanations

1) Application

The present application is based on a method for producing virus-like particles by the assembly or *in vitro* assembly of capsomers of the polyomavirus. Biologically active macromolecules which are bonded to charged amino acids of the capsomers are packaged in the presence of non-ionic stabilisers.

2) Prior art

Reference is made to the following documents:

D1: DE-A-19952957

D2: WO-A-0142780

D3: McCarthy et al., Journal Of Virology, The American Society For Microbiology, US (1998), 72(1), 32-41

D4: WO-A-0057906

D5: Salunke et al., Biophysical Journal, New York, US, (11-1989), 56, 887-900

D6: Colomar et al., Journal Of Virology, New York, US, (01-05-1993), 67(5), 2779-2786

3) Novelty (PCT Article 33(2))

3.1 D1 describes the production and assembly of virus-like particles from the polyomavirus capsid protein VP1. Assembly is carried out following dialysis of the recombinantly produced protein against 10 mM HEPES, 50 mM NaCl, 0.5 mM $CaCl_2$, 5% glycerol and a pH of 7.2 for 72 hours at room temperature (see page 10, lines 26 to 29).

3.2 D1 also describes the packaging of DNA in the virus-like particles in a solution with a dialysis buffer with 20 mM sodium acetate, a pH of 5.0, 100 mM NaCl, 1 mM $CaCl_2$ and 5% glycerol for 4 days at room temperature (see example 10, page 13, lines 46 to 52).

3.3 D1 thus discloses all the essential technical features of claims 1, 3 and 4 (assembly of polyomavirus capsomers, DNA as packaged macromolecule, polyol as non-ionic stabiliser) and therefore prejudices the novelty of these claims.

4) Inventive step (PCT Article 33(3))

4.1 Claim 2 is directed to a method according to claim 1, in which the assembly is carried out in a buffer with an ionic concentration of less than 250 mM and in the presence of an oxidising redox system.

4.2 Document D1, which is regarded as the closest prior art, discloses the production of virus-like particles for packaging macromolecules. The method described by D1 differs from the subject matter of

claim 2 in that no oxidising redox system is used.

4.3 The present invention can therefore be considered to address the problem of developing an alternative method for the assembly of polyomavirus capsomers and for packaging macromolecules such as DNA.

4.4 Other methods described in the prior art for the assembly of polyomavirus capsomers mention the removal of reducing components from the preceding disassembly stage as an essential prerequisite for reassembly (D3: page 35, left-hand column, third paragraph to right-hand column, second paragraph; D2: examples 3 to 5, pages 46 to 53). D2 proposes, for reassembly with a molecule to be packaged, in addition to removing the sulfhydryl reducing agent, even adding an excess of oxidising agent (page 22, lines 16 to 20; claim 46). According to D2 and D3, however, the reassembly takes place with higher salt concentrations (0.5 M NaCl) and thus with higher ionic concentrations in order to stabilise the particles (D3: page 32, left-hand column, fourth paragraph; D2: example 3, page 46). The methods described in D4 and D5 are also based on high salt concentrations. According to the method as per D6, SV40 capsid proteins are assembled by reducing the ionic concentration, this being carried out, however, in the presence of a reducing agent.

4.5 In D1, modified coat proteins, for example fusion proteins without cysteines, are also used. The addition of a redox system appears to not be necessary. A combination of the methods specified in D1 with the other prior art methods allows for numerous variations in respect of various

parameters, for example pH level, ionic concentration, stabilisers and redox conditions. Therefore, the specific combination of stabiliser, ionic concentration and redox conditions proposed in the present application appears not to be suggested.

4.6 Consequently, the subject matter of claim 2 involves an inventive step and thus meets the criterion in PCT Article 33(3).

4.7 Claims 3 to 5 are worded such that they contain the subject matter of claims 1, 2, 3 **or** 4 and supplement these claims with additional technical features. The subject matter of these claims could be considered inventive only if it were to include the features of claim 2, i.e. if the reference to claim 2 were not merely optional.